Morphological and Molecular Identification of *Pythium aphanidermatum* Causing Root Rot of Tomato in a Hydroponic Substrate Culture in the South Western Region of Japan

Motoaki Tojo, Shohei Matsuura*, Motoki Takase and Lorien E. Radmer
(Graduate School of Life and Environmental Sciences, Osaka Prefecture University,*
*Hiroshima Prefectural Technology Research Institute, Agriculture Research Center)

*Pythium aphanidermatum* (Edson) Fitzpatrick is a major causal agent of root rot and wilt of tomato (*Solanum lycopersicum* L.) all over the world[3,10]. The pathogen can cause severe problems in hydroponically grown tomatoes[5]. Despite economic losses of hydroponically grown tomatoes due to *P. aphanidermatum*, no accurately identified isolates of the pathogen from these conditions are available. Here we describe the morphological and molecular characteristics of *P. aphanidermatum* isolates recovered from the rotted roots of tomatoes grown in a hydroponic substrate culture in the south western region of Japan in order to provide public materials for the study of the pathogen.

**Materials and Methods**

Damaged tomato plants were collected from a commercial hydroponic substrate culture in Hiroshima Prefecture, the south western region of Japan in August, 2012. The disease first appeared in August 2012. Approximately 2000 plants in the culture system were damaged by the disease. The sections of rotted roots were washed in tap water, air dried and incubated on water agar (1.5%) at 25°C. *Pythium* mycelia on the plates were cultured on corn fish meal agar prepared according to Tojo et al.[6] until use. The *Pythium* isolates were grown in a grass blade culture prepared according to Martin[3]. Morphological identification was based on the keys of van der Plaats-Niterink[9]. Hyphal growth rates at different temperatures were determined on potato carrot agar (PCA) according to the method described previously by Tojo et al.[6]. Molecular identification was performed on a representative isolate. DNA extractions, amplification and sequence reactions of the internal transcribed spacer (ITS) region of rDNA and mitochondrial cytochrome oxidase 2 (*cox2*) gene of the isolates were performed according to the method described by Uzuhashi et al.[7,8]

**Results and Discussion**

A total of eleven *Pythium* isolates recovered from rotted roots of tomatoes grown in a hydroponic culture showed similar morphology and growth temperature to each other. Based on their morphology, all isolates were identified as *P. aphanidermatum*. The detailed descriptions here are of the representative isolate OPU854 (= NBRC 109473, MAFF 243776).

*P. aphanidermatum* isolate OPU854: Primary hyphae were up to 10 μm wide. Sporangia were mostly terminal, occasionally intercalary, consisting of inflated filamentous structures or complexes of swollen hyphal branches (Fig. 1A). Diameter of encysted zoospores ranged from 10.0–13.5 μm (Fig. 1B and C). Oogonia were terminal and 18.0–30.0 (ave. 23.6) μm in diameter (Fig. 1D). Antheridia were terminal or intercalary, 1 per oogonium, monoclinal or dircinal (Fig. 1D). Breadth of antheridial cells ranged from 7.0–12.5 μm. Oospores were aplerotic and 13.5–24.0 (ave. 20.3) μm in diameter (Fig. 1D). Thickness of the oospore wall ranged from 1.0–2.0 (ave. 1.6) μm. Cardinal temperatures for hyphal growth on PCA were 10°C minimum, 37°C optimum, and 40°C maximum. Daily growth rate at 25°C was 31.9 mm per day.

The ITS sequence of the representative isolate OPU854 was identical (100%) to those of *P. aphanidermatum* isolate CBS118.80, which was used for the species description by van der Plaats-Niterink[9]. The *cox2* gene sequence of the

---

Corresponding author: Motoaki Tojo (E-mail: tojo@plant.osakafu-u.ac.jp)
Accepted 23 January, 2013
isolate also matched (99.5%) that of *P. aphanidermatum* isolate ATCC 96212\(^1\). These sequence have been deposited in the DDBJ/EMBL/GenBank database under accession numbers KC438411 for the ITS region and KC438410 for the *cox2* gene.

Accurate identification of the pathogen is required in order to develop new control measures for plant diseases. Despite the economic importance of *P. aphanidermatum* on hydroponically grown tomatoes, there was no public material of the pathogen. The present *P. aphanidermatum* isolate which has been morphologically and molecularly identified will be useful in testing control measures for the disease occurring in hydroponically grown tomatoes.

**Fig. 1.** Morphology of *Pythium aphanidermatum* isolate OPU854 formed in a grass blade culture. A Sporangium. B Encysted zoospores. C Germinated zoospores. D Terminal oogonium and aplerotic oospore with intercalary antheridium. Bar 20 \(\mu m\).

**References**

**Summary**
Isolates of *Pythium aphanidermatum* from the rotted roots of tomatoes grown in a hydroponic substrate culture in the south western region of Japan were characterized based on morphology, hyphal growth temperature, and the sequences of their ITS-rDNA and mitochondrial *cox2* gene regions.